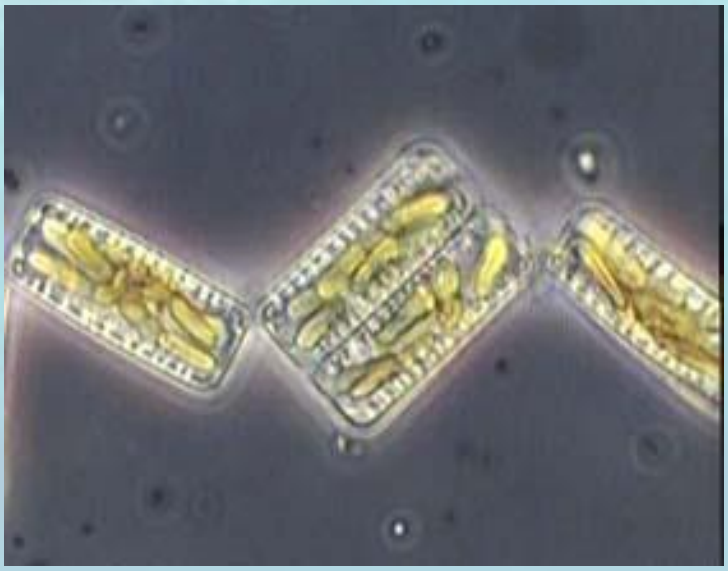
SCIENCE FOCUS: Fluorescence

Fluorescence – That Healthy Glow



The diatom *Rhabdonema minutum*. The chloroplasts are the ovoid bodies contained in each of the rectangular cells. (The cell walls (frustules) of diatoms are composed of silica, SiO_2. Chloroplasts contain the photosynthetic pigment chlorophyll, used for photosynthesis. Image courtesy of The Baltic Sea Portal – Algaline.

First let's state the obvious, as a short review: the fundamental goal of ocean color remote sensing has been to measure the concentration of the pigment named chlorophyll, which is contained in phytoplankton (see image above). Chlorophyll absorbs light energy as part of the process of photosynthesis, which allows plants (phytoplankton are floating plants) to synthesize organic carbon (carbohydrates, actually) from carbon dioxide (CO_2) and water (H_2O).

But now, with increasingly sophisticated sensors, better data, and improved algorithms, ocean color remote sensing is addressing its next fundamental goal: accurately determining phytoplankton primary productivity, which is how much organic carbon the phytoplankton produce.

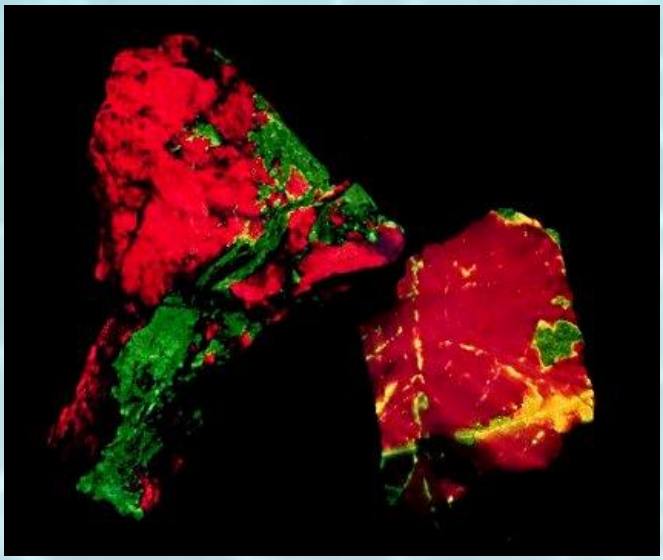
So measuring the concentration of chlorophyll in phytoplankton is Step 1, and determining how much organic carbon these phytoplankton are producing is Step 2. It's quite a way from Step 1 to Step 2.

The basic way that the concentration of chlorophyll is determined is to measure how much light at a particular wavelength (443 nm) is absorbed. Analytical algorithms developed by ocean optical research convert this measurement to chlorophyll *a* concentration. There are other chlorophyll molecules, called *b, c, d, and e.* But chlorophyll *a* is the most prevalent and the most important one.

Getting to Step 2 requires some local knowledge: the type of phytoplankton living in a given area, how much chlorophyll they contain, how much light is available for photosynthesis (called Photosynthetically Active Radiation, or PAR), and also how "lively" the phytoplankton are. Are they young and marvelously efficient, converting light to carbon eagerly and rapidly? Or have they been a bit overexposed and become tired, so that their efficiency is reduced?Or are they exhausted and ready to sink to the bottom?

(Pardon our anthropomorphisms.) Phytoplankton do vary significantly in their ability to convert light to carbon (lengthy exposure to light can reduce their efficiency, for example) and this variability is directly related to their physiological state. What is required is a remote sensing method for determining the physiological state of phytoplankton.

It may be difficult to believe that a satellite instrument observing the Earth's oceans from an altitude of hundreds of kilometers could determine the health of individual, microscopic phytoplankton cells. But that's where **fluorescence** comes in. Many substances, both organic and inorganic, release light of one wavelength when they are exposed to light of another wavelength. Below is a picture of the minerals calcite and willemite under ultraviolet (UV) light. The minerals absorb light in the UV range and releases light in the visible range—green for willemite, red for calcite.



Besides minerals, complex organic molecules are frequently fluorescent. If you are wearing a shirt made of nylon and you stand in a UV light, the nylon will glow brightly. In this picture, even the teeth of the subjects are fluorescent!

This image and the preceding image courtesy of Thomas S. Warren Museum of Fluorescence, Ogdensburg, New Jersey, USA.

Chlorophyll is also a complex organic molecule. Happily, chlorophyll does fluoresce, though pretty weakly, when exposed to light. Even better, chlorophyll fluorescence can be used to determine the physiological state of phytoplankton.

However, in order to be useful for remote sensing, a sophisticated instrument with a band located at the proper wavelength is required. SeaWiFS, designed as a global ocean color mission, does not have the necessary band. But MODIS, the Moderate Resolution Imaging Spectroradiometer, does. The fluorescence band is one reason that MODIS is the next step in ocean color remote sensing. SeaWiFS data are used to determine chlorophyll *a* concentration, and provided with those few assumptions, initial estimates of primary productivity can be made. MODIS data will allow more refined estimates of primary productivity by incorporating data on the physiological state of the primary producers themselves.

How it works (in theory, at least):

A simple definition of fluorescence: emitted radiation that is released from a substance when the emission is stimulated by exposure to electromagnetic radiation. In fluorescent lights, the gas inside the light is stimulated by electricity. For fluorescent minerals, the solid mineral is stimulated by UV radiation.

At the molecular level, the description of what happens goes like this: a substance absorbs a photon of electromagnetic radiation, which causes an electron to move from a low energy state to a higher energy state. When the electron returns to a lower energy state, a photon is emitted. This photon is fluorescent radiation.

For chlorophyll, the situation is a bit more complicated. First it should be noted that many photosynthetic organisms have their chlorophyll contained in structures called "chloroplasts". See the image at the top of this page for a good view of chloroplasts. The light energy absorbed by the chloroplast first excites pigment molecules of light harvesting chlorophyll (LHC) proteins. These proteins transfer their energy to one of two photosynthetic systems, called Photosystem I (PS-I) or Photosystem II (PS-II).

Each of these photosystems are contained in "reaction centers" that contain the pigments to convert the light energy to an oxidation and reduction potential that drives dark electron transport. The reaction centers can only accept a finite number of photons in a given period of time, and excess energy absorbed by the pigments must be released.

One of the ways that the energy escapes is fluorescence (another way is as heat, which is absorbed by photoprotective pigments). Approximately 3-9% of the light energy absorbed by chlorophyll pigments is re-emitted as fluorescence. The fluorescent light is at a longer wavelength than the absorbed light. The fluorescence peak for chlorophyll *a* is at 683 nm.

It's also important to note that the light energy absorbed by the photosynthetic pigments drives electron transport through both the PS-I and PS-II systems. This electron transport causes the oxidation of water, oxygen evolution, the reduction ofnicotine adenine dinucleotide phosphate (NADP+) to NADPH (a hydrogen atom is added), membrane proton transport, eventually leading to the synthesis of adenosine triphosphate (ATP), a key molecule in cellular energy systems. The loss of light energy as fluorescence comes primarily from the PS-II system.

So chlorophyll fluorescence and photosynthesis are connected to one another, and that connection is what enables the use of fluorescence to determine the photosynthetic efficiency of phytoplankton, i.e., how productive they are.

We won't go into detail about the PS-1 and PS-II systems, but we'll simply provide this link to a detailed description of photosynthesis, which includes schematic diagrams of each photosynthetic system: Photosynthesis.

What we want to concentrate on now are two topics: 1) how fluorescence is related to phytoplankton primary productivity and 2) the data that MODIS provides that can be used for this purpose.

Fluorescence and primary productivity

Most current models of primary productivity rely on estimation of light absorption by phytoplankton, because that is what ocean color sensors have been capable of measuring. However, phytoplankton vary considerably in their ability to utilize light energy. Different species of phytoplankton utilize light differently, and even single species utilize light differently at different stages of their life cycle and under changing environmental conditions.

In contrast, the use of fluorescence data may provide a more direct way of determining the efficiency of light utilization in phytoplankton. **The amount of fluorescent radiation that is released by phytoplankton exposed to light is related to the amount of light that is utilized by the phytoplankton for photosynthesis.** Quantifying that relationship is still a challenge to researchers.

Another term for the amount of fluorescence released by chlorophyll is the "fluorescence quantum yield", $\mathbf{F_f}$. The amount of fluorescence measured is related to F_f by this relationship:

$$F = [chl]\,[PAR\ a^*]\,F_f$$

where F is chlorophyll fluorescence,
[chl] is chlorophyll concentration,
PAR is Photosynthetically Active Radiation,
and **a*** is the specific absorption coefficient.

One of the most important factors governing phytoplankton primary productivity is exposure to light. Darkness provides a chance for the photosynthetic systems to "rest" and regenerate, so that photosynthetic efficiency, and fluorescence, will be highest when the phytoplankton are first exposed to light. As the photosynthetic reactions take place, the systems use the available chlorophyll to produce carbohydrates, and their efficiency declines because some of the energy is lost as light (fluorescence) and also as heat, and because the molecular reaction sites within each chlorophyll molecule are slowly used up. Another factor is the production of photoprotective pigments, which are created to act as a "sunblock" in phytoplankton that are exposed to high light conditions. These pigments absorb light but do not pass it on to the PS-II system. The creation of photoprotective pigments means that less energy is devoted to photosynthesis in the cells of phytoplankton, so they are less efficient.

Thus, phytoplankton cells that are "young" and which have not recently been exposed to light will have a much greater photosynthetic efficiency than older cells which have been exposed to light for a period of time. Relating these physiological differences to the measured fluorescence and calculating the effect on primary productivity is the goal of algorithms using MODIS fluorescence data.

MODIS Fluorescence Data

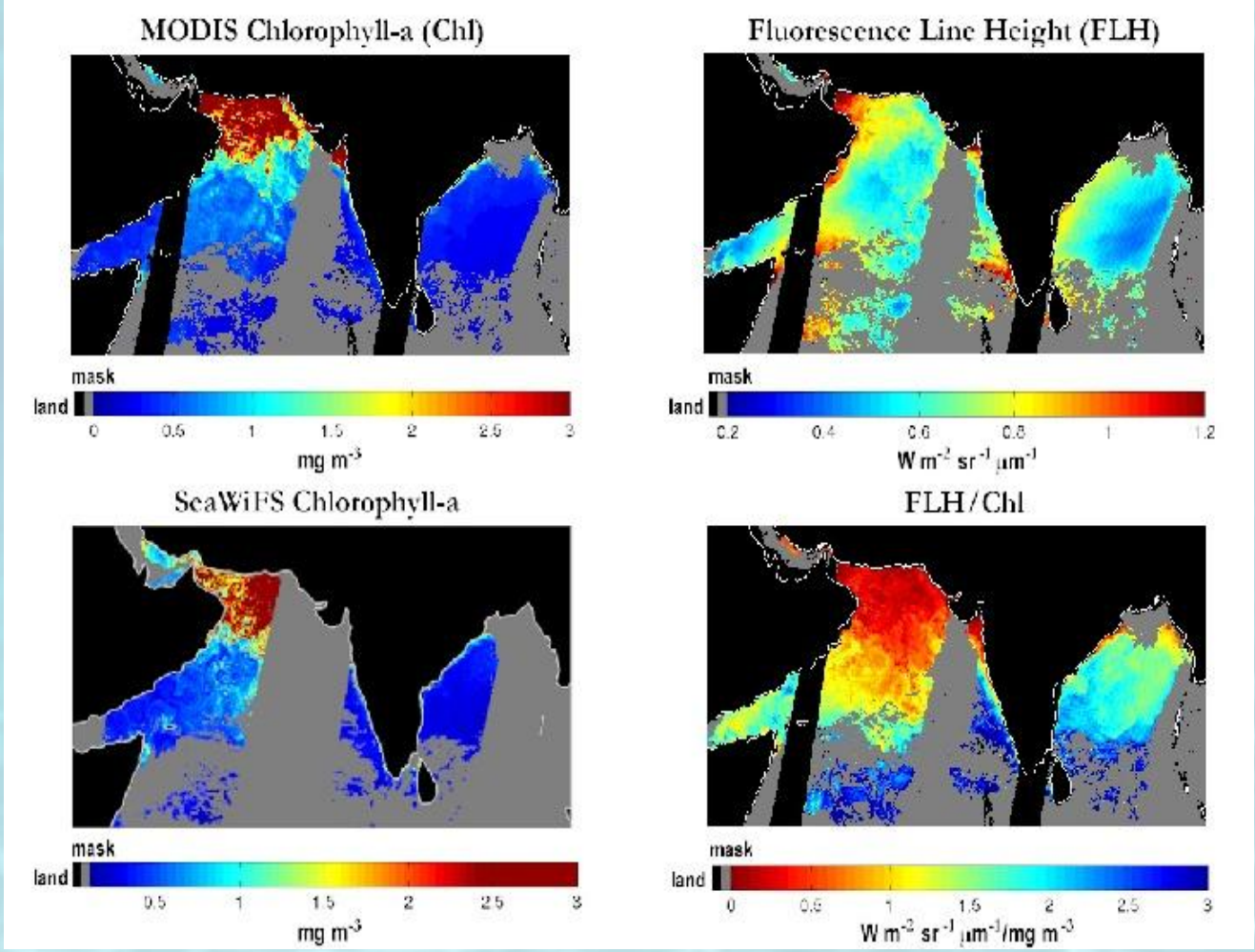
The MODIS band that is used for the measurement of chlorophyll fluorescence is band 14, located at 676.7 nm. Ideally, the band would be located at the fluorescence peak wavelength of 683 nm, but oxygen molecules in the atmosphere absorb strongly at 687 nm, and this would interfere with the detection of the fluorescence signal.
Data from MODIS (the 676.7 nm band) is converted into three different parameters:

Chlorophyll fluorescence line height (FLH)
This data product indicates the intensity of chlorophyll fluorescence.

Chlorophyll fluorescence baseline
This data product establishes the background water-leaving radiance at the fluorescence band. The algorithm to establish the chlorophyll fluorescence baseline uses the measured water-leaving radiances for bands13 and 15, 665.1 and 746.3 nm, respectively.

Chlorophyll fluorescence efficiency (CFE)
This data product indicates how much incoming light (PAR) is being converted to fluorescent radiation. Ultimately, CFE will be related to the photosynthetic efficiency, and hence the primary productivity, of phytoplankton.

The image below shows a comparison of chlorophyll *a* measured by SeaWiFS and MODIS, FLH from MODIS, and the FLH/chlorophyll concentration ratio, for the Arabian Sea and the Bay of Bengal on March 1, 2000. Dr. Mark Abbott, Dr. Ricardo Letelier, and Jasmine Nahorniak of Oregon State University created this image display. Dr. Abbott's research group at Oregon State is responsible for the calculation of the MODIS chlorophyll fluorescence parameters and the refinement of primary productivity estimates using chlorophyll fluorescence data.



Acknowledgments:
We thank Dr. Mark Abbott for his review of this *Science Focus!* article.

Links:

Algorithm Theoretical Basis Document: Chlorophyll Fluorescence (MODIS Product Number 20) (PDF)

Phytoplankton Fluorescence from MODIS (PDF)

Chlorophyll Fluorescence as a Biological Indicator of Primary Productivity (PDF)